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(54) METHOD AND APPARATUS FOR SEPARATING POLYNUCLEOTIDES USING MONOLITHIC CAPILLARY COLUMNS

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(57) ABSTRACT

Methods and devices based on capillary monolithic columns, preferably consisting of an underivatized poly(styrene-divinylbenzene) monolith, for separating a mixture of polynucleotides by ion pair-reverse phase-high performance chromatography (IP-RP-HPLC). In various aspects of the method and device the monolith is characterized by one or more of the following: the monolith is contained within a capillary tube; the monolith is immobilized by covalent attachment at the inner wall of the tube; the tube is devoid of retaining frits; the monolith is characterized by having above 10,000 theoretical plates per meter and preferably above 200,000 theoretical plates per meter; the method uses a mobile phase which is devoid of EDTA; the monolith has a surface morphology that is rugulose or brush-like; the chromatographic surfaces of the monolith are non-porous: the monolith has channels sufficiently large for convective flow of the mobile phase; the monolith is formed from a polymerization mixture including underivatized styrene, a crosslinking agent, and a porogen, wherein the porogen includes tetrahydrofuran. The monolith can be incorporated into a miniaturized chromatography system which can be coupled to a mass spectrometer for on-line separation and mass determination of single- or double-stranded polynucle-

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